

U198US(PCT)

**METHOD AND DEVICE FOR DISINFECTING A MICROTOME CRYOSTAT****Description**

The invention concerns a method for disinfecting a microtome cryostat, comprising a defrosting phase, provision of a vaporous disinfectant which acts upon the closed cryostat chamber, and a period of time during which the disinfectant is effective.

The invention also concerns a device for disinfecting a microtome cryostat, comprising a microtome in a cryostat chamber, a refrigerator, a means for providing a vaporous disinfectant, and a disinfectant control means to set an effective time after a defrosting phase.

Disinfection is necessary to protect the operating personnel since microtomes are used for cutting tissue samples which are often infected with germs. Spraying is problematic, in particular, for cryostat microtomes, since the moisture must be removed from the cryostat chamber. Residual moisture on a cryostat microtome freezes after refrigeration for the next working step. Guidances etc. may thereby freeze with the consequence that the microtome is inoperable, or liquid disinfectant, e.g. a highly concentrated alcohol solution, remains in the chamber and causes further problems, even danger of explosion.

US 2002/0139124 A1 proposes a solution to this problem by disinfection with ozone. This is, however, disadvantageous, since ozone is highly corrosive and damages the surfaces via oxidation. A further disadvantage is that residual ozone is difficult to remove, is poisonous and highly

inflammable, and can partially escape from the device even during the disinfecting phase, which could endanger the operating personnel.

The company leaflet "AS 600 Cryotome" (ANGLIA SCIENTIFIC) proposes another solution for decontamination using UV radiation.

Disadvantageously, the UV radiation does not reach the shadow regions. Moreover, the UV radiation cannot penetrate deeply enough into cutting waste or sample residues nor into microscopically small depressions in metal surfaces. This method of decontamination is therefore unsatisfactory.

A proposal of the above-mentioned type is disclosed in the company leaflets "AS 620 Cryotome" and "AS 620 Cryotome Instruction Manual" (ANGLIA SCIENTIFIC Instruments LTD). A formalin dispenser is thereby heated and the closed cryostat chamber is charged with formalin vapor. The above-mentioned problem of residual moisture in the cryostat and, in particular, on the microtome, also occurs in this case with the consequence that the device must be left open for a relatively long time to dry. Disinfectant thereby escapes which should be prevented for health reasons and due to possible annoyance caused by bad smell. Moreover, the device is inoperable for a relatively long time or is not completely dried.

It is therefore the underlying purpose of the invention to further develop a method and a device of the above-mentioned type to ensure rapid and effective disinfection to permit as fast a renewed operation of a microtome cryostat as possible as well as complete drying thereof.

This object is achieved in accordance with the inventive method in that a temperature difference is generated in the cryostat chamber after the

effective time and disinfectant deposited in the colder region is discharged.

This object is achieved in accordance with the inventive device in that the control is designed to generate a temperature difference in the cryostat chamber after the effective time through heating and/or cooling, and a collecting device is disposed in the colder region to remove deposited disinfectant.

With the present invention, disinfectant and moisture deposit in a colder region after disinfection and are discharged from there in a controlled manner, thereby permitting fast drying and rapid renewed operation while subjecting critical regions of the device, such as the microtome, to particularly effective drying by heating instead of cooling them. This prevents formation of ice during renewed cooling for the next working step which would otherwise impair or prevent further operation. In particular, the device is ready for reliable reuse within a short time after disinfection.

The most different means can be used as disinfectant, mainly in the form of aqueous solutions, e.g. glutaraldehyde, benzalconium chloride, formalin and didecyl dimethyl ammonium chloride.

The temperature difference can be generated both by cooling as well as by heating of a region. It is thereby advantageous to use existing cooling or heating devices. The temperature of the refrigerator of the cryostat may be reduced below 0°C in a cooling phase after the effective time until at least the majority of the disinfectant has deposited on the refrigerator followed by defrosting of the refrigerator to discharge the disinfectant from the cryostat chamber using a collecting device. A collecting device of this type is usually disposed below the refrigerator to

be able to remove the defrosted dew or defrosted ice liquids from the cryostat chamber.

Another possibility is to heat the microtome after the effective time. Due to such heating, the disinfectant, e.g. the disinfecting solution, is transferred from the microtome to colder parts of the device with the result that the microtome is dried for the subsequent cooling phase. The temperature difference can, of course, be obtained through cooling as well as heating, wherein a combination of the two above-mentioned measures is preferred. The colder regions within the cryostat chamber are preferably selected such that the deposited disinfectant or moisture can be easily collected and discharged.

In any event, the heating temperature is preferably considerably higher than the surrounding temperature of the cryostat in order to completely dry the microtome.

To also achieve maximum effective disinfection, the invention proposes blowing the vaporous disinfectant into the cryostat chamber. This forced convection causes uniform wetting of all components. The disinfectant can be vaporized through a heater, wherein vaporization is preferably effected through ultrasound which permits generation of a uniform aerosol without separating the phases of the substances of the disinfectant, thereby providing uniform disinfection. The disinfectant may, of course, also be present in the form of vapor.

The cryostat is preferably heated after the defrosting phase to at least the surrounding temperature. This heating is preferably followed by a temperature balancing time to ensure that all components have the same temperature and are subjected to uniform disinfection during the effective time of the disinfectant. Different heaters may be used for

heating, wherein a heater installed in the microtome is preferred. Heating via the microtome prevents thawing thereof and thereby dilution of the solvent during the actual subsequent disinfection during the effective time, which provides excellent disinfection of the microtome.

The cutting waste should be mechanically removed before introduction of the vaporous disinfectant. This may be effected manually or the cutting waste may be suctioned to thereby prevent residual contamination in the cryostat chamber by the cutting waste.

Moreover, vaporous disinfectant may be suctioned into a suction system for disinfection thereof. Suctioning disinfects tubes, filters, pump and blocking devices of the suction system.

Further developments and embodiments of the device may correspond to the further developments and embodiments of the method and vice versa, wherein the above-mentioned advantages can be achieved in each case.

In a further development of the device, the control is designed to reduce the temperature of the refrigerator of the cryostat below 0°C in a cooling phase after the effective time until at least the majority of the disinfectant has deposited on the refrigerator, and the refrigerator is subsequently thawed to discharge the disinfectant from the cryostat chamber using the collecting means.

Alternatively or additionally, the microtome may comprise a heater and the control may be designed to initiate heating of the microtome after the effective time thereby completely drying the microtome as already described in connection with the method.

The refrigerator also advantageously comprises a heater with the control being designed to switch on the heater to accelerate thawing. The disinfectant and moisture sublimated on the refrigerator can thereby be easily liquefied and discharged, ensuring rapid reoperation of the device.

The means for providing a vaporous disinfectant preferably comprises a blower for introducing the vaporous disinfectant into the cryostat chamber to achieve forced convection into the cryostat chamber with uniform distribution of the disinfectant. The means for vaporizing the disinfectant is preferably provided with an ultrasound actuator to realize the above-mentioned atomization of the disinfectant without phase separation. The means for generating the vaporous disinfectant is preferably supplied with disinfectant from a supply container. A valve may be provided to control the liquid level of the disinfectant in the means for generating the vaporous disinfectant.

The invention is explained below with reference to the figures of the drawing showing examples.

Fig. 1 shows an exemplary view of the temperature dependences and method steps of an inventive method; and

Fig. 2 shows an embodiment of an inventive microtome cryostat.

Fig. 1 shows examples of possible temperature dependences of the inventive method, wherein the temperatures  $T$  in °C are plotted against time  $t$  and possible method steps 11 through 19 are shown.

Starting from an operational temperature of the microtome of between -5°C and -35°C, a defrosting phase 11 is initiated with heating 12 to at least the surrounding temperature  $T_U$  which is within a range from 20 to

25°C. This process is preferably accelerated by operating the heater 7 of the microtome 6, wherein heating via the microtome 6 is advantageous in that the latter does not thaw. For this reason, the disinfectant 2 cannot be diluted by the dew during subsequent disinfection. In view of the device features, reference is made to Fig. 2.

Due to the microtome heater, the temperature  $T_M$  of the microtome 6 increases faster than the temperature  $T_K$  of the cryostat chamber 3. Heating 12 is therefore preferably followed by a temperature balancing time 12'. When all components have reached approximately the same temperature, a vaporous disinfectant 2 is provided 13 which is blown into the cryostat chamber 3.

When all components have been uniformly wetted with disinfectant 2, heating 15 of the microtome 6 by the microtome heater 7 is initiated after lapse of an effective time 14, thereby further increasing the temperature  $T_M$  of the microtome 6, e.g. to 50°C to transfer moisture and disinfectant from the microtome 6 to the colder parts of the cryostat chamber 3. To cause precise sublimation of the disinfectant on the refrigerator 4 of the cryostat 1, the cryostat 1 is cooled below 0°C in a cooling phase 16, preferably to a region of -10°C. Temperature differences  $\Delta T_1$  and  $\Delta T_2$  are thereby generated, wherein  $\Delta T_1$  is the difference between the temperature  $T_M$  of the microtome 6 and of the temperature  $T_V$  of the refrigerator 4, and  $\Delta T_2$  is the difference between the temperature  $T_K$  of the cryostat chamber 3 and the temperature  $T_V$  of the refrigerator 4. In consequence thereof, the disinfectant 2 and moisture on the refrigerator 4 are sublimated on the refrigerator 4 and deposit there in the form of dew and ice. A defrosting phase 17 of the refrigerator 4 is then initiated during which the thawed liquid is discharged. The thawed liquid is discharged 18 using a collecting device 5 which guides the thawed liquid out of the cryostat chamber 3. Thawing

17 of the refrigerator 4 is suitably supported by a heater 10 of the refrigerator 4, wherein the latter is heated e.g. to +6°C. When the thawed liquid has been discharged, the cryostat chamber 3 and, in particular, the microtome 6 are dry. Cooling can be repeated for reoperation 19 to bring the microtome cryostat 1 again to its operating temperature for new object processing.

Fig. 2 shows an embodiment of an inventive microtome cryostat 1. The microtome cryostat 1 consists of a cooled housing which surrounds a cryostat chamber 3 and can be opened via an opening 32. The cryostat chamber 3 contains a microtome 6 including an object carrier 33 and a knife 34 for producing the cuts. The cryostat chamber 3 is cooled using a refrigerator 4, e.g. a vaporizer, which is connected to a coolant supply 30. The microtome 6 as well as the refrigerator 4 are provided with heaters 7 and 10 to provide rapid thawing and heating. A collecting means 5 is provided below the refrigerator 4 which is designed e.g. as drip pan which discharges thawed liquid from the cryostat chamber 3 into a supply container 28 via an outlet 25.

Moreover, a means 8 for providing a vaporous disinfectant 2 blows the evaporated disinfectant 2 via a pipe into the cryostat chamber 3 in the direction of the arrows 35. Towards this end, a blower 20 and an ultrasound actuator 21 are provided to generate the aerosol from the liquid disinfectant 2. A supply container 22 and a valve 23 thereby ensure that the liquid level 24 of the disinfectant 2 in the means 8 is always maintained.

The invention also comprises a control 9 which is connected to the heater 7 of the microtome 6, to the heater 10 of the refrigerator 4, to the ultrasound actuator 21, the blower 20 and the coolant supply 30 of the refrigerator 4 via connecting lines 31. The control 9 may furthermore be

connected to sensors (not shown) for detecting the temperature, air moisture, etc. to ensure effective control of the described temperatures and drying processes. Connection to a ventilator 29 of a suction system 26 and to a valve 23 and further functional elements is also possible.

The connecting lines 31 provide control 9 of the temperature dependences  $T_K$ ,  $T_M$  and  $T_V$  (Fig. 1) and of the method steps 11 through 19 by controlling the heaters 7 and/or 10 and cooling using the refrigerator 4. The control 19 also controls the supply 13 of the vaporous disinfectant 2 via the means 8.

The control 9 can also initiate suctioning of vaporous disinfectant 2 into the suction system 26 for disinfecting e.g. the ventilator 29, the pipes and the filter 27 located therein.

The illustrated temperature dependences  $T_K$ ,  $T_M$  and  $T_V$  and working steps 11 through 19 of Fig. 1 and the device features of Fig. 2 are only examples. The temperature differences  $\Delta T_1$  and  $\Delta T_2$  for the deposit of disinfectant 2 and moisture and the discharge thereof are essential. An alternative design of the heaters or alternative generation of temperature differences within the cryostat chamber 3 are also possible, e.g. only through heating or only through cooling, or the time sequence may be different, wherein e.g. method steps 11 through 19 could partially overlap.

**Method and Device for Disinfecting a Microtome Cryostat****List of Reference Numerals**

- 1 microtome cryostat (cryostat)
- 2 disinfectant
- 3 cryostat chamber
- 4 refrigerator (of the cryostat) e.g. vaporizer
- 5 collecting device
- 6 microtome
- 7 microtome heater
- 8 means for providing a vaporous disinfectant
- 9 control
- 10 heater of the refrigerator
- 11 defrosting phase
- 12 heating to at least surrounding temperature
- 12' temperature balancing time
- 13 providing a vaporous disinfectant
- 14 effective time of the disinfectant
- 15 heating the microtome
- 16 cooling refrigerator
- 17 thawing refrigerator
- 18 discharging the thawed liquid
- 19 cooling for reoperation
- 20 blower
- 21 ultrasound actuator
- 22 supply container for disinfectant
- 23 valve
- 24 liquid level
- 25 discharge
- 26 suction system

- 27 filter
- 28 supply container for thawed liquid
- 29 ventilator
- 30 coolant supply of the refrigerator (vaporizer)
- 31 connecting lines for control
- 32 opening of the cryostat chamber
- 33 object carrier
- 34 knife
- 34 arrows: blowing in vaporous disinfectant

T temperature in °C

t time

$\Delta T_1$  temperature difference between refrigerator and microtome

$\Delta T_2$  temperature difference between refrigerator and cryostat chamber

$T_u$  surrounding temperature of the cryostat

$T_k$  temperature of the cryostat chamber

$T_m$  temperature of the microtome

$T_v$  temperature of the refrigerator (vaporizer)